

## THE ULCER DISEASE (BLACK OPHTHALMIA?) OF RAINBOW TROUT.

(*Micrococcus pyogenes*.)

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During the months of February and March of this year a disease appeared among the rainbow trout, *Salmo irideus*, in the fish hatchery of the New South Wales Government at Prospect. The hatchery adjoins the reservoir of the Sydney water supply, and under normal conditions is furnished with a continuous supply of pure water. Owing, however, to the drought the water in the reservoir fell very low, and the tanks in the hatchery did not receive an adequate supply. As a result the water in the tanks, which were carrying a maximum number of one, two and three-year old trout, became foul, and there can be little doubt that this was indirectly responsible for the outbreak of the disease. When the supply again became regular the disease almost disappeared.

The disease is characterised by the fishes having a sluggish, aimless movement in the water, in which they generally keep near the surface. The snout and tail become whitish from their rubbing the snout against the sides of the cement tank, and at other times resting vertically in the water and moving the tail to and fro against the cement tank bottom. When the eye becomes affected the lens whitens and the eye decays, leaving an empty capsule. The epidermis generally becomes dark in colour, and this, in conjunction with the atrophy of the eye, has given rise to the name of the disease, black ophthalmia.

Other fishes develop small white ulcers on various parts of the epidermis. Beneath the skin and under the sores the muscle is hæmorrhagic. The scales fall off from these places, the epidermis disappears, and the muscle becomes exposed. This has a reddish colour, while the epidermis at the margin for about one-sixteenth of an inch is white, so that the combination of colours gives the sore a striking appearance. Although these are of the nature of ulcers there is no appearance of pus. It is possible that the black ophthalmia and the production of these ulcers are due to two different causes, but at the beginning of the investigation it was believed that only one disease was affecting the trout.

The post-mortem examination of many carcasses that I received showed that the body organs were apparently healthy, and that they were practically sterile. Neither moulds nor algæ could be detected on the ulcers, in the orbital capsule or on the partially affected eye. The muscles and organs when examined in the fresh condition were free from parasites. But under the small unbroken ulcers coccoid forms, occurring singly and in groups, were detected, and from these a pure culture of a white micrococcus which had all the cultural and other characters of *Micrococcus pyogenes*, (Rosenbach) Migula, was obtained.

In mammals this bacterium is responsible for the formation of ulcers, boils, etc., containing pus, and the presence of pus may be said to be the only difference between the ulcers of the trout and those of the higher vertebrates. As the pyogenic bacteria are not known to produce pus in the epidermal lesions of fishes, it seemed probable that the disease would be reproduced by inoculating the micrococcus into healthy trout.

To test this point Mr. J. A. Brodie, of the Department of Fisheries, enabled me to infect several trout at the Prospect hatchery. The inoculations took place some time after the isolation of the bacterium, because it was not until about two months had elapsed that a constant supply of water could be obtained. Six healthy trout were inoculated with a suspension of the micrococci in normal saline under the epidermis of the posterior region, between the median and dorsal lines. After the

operation the fishes were put into metal tubs, fed with a stream of fresh water from an overhead tap, and the tubs were protected by nets placed over them. The trout were left in charge of the caretaker, who had instructions to forward at once to Sydney the carcasses of any fish that might die, as delay in doing so might seriously influence the investigation.

On the sixth day after inoculation one of the trout died, and the carcass was received at the laboratory. The caretaker had noted that it had been sickly on the previous two or three days. The carcass had no epidermal lesions, and on dissection the organs were found to be healthy. The blood vessels of the stomach, intestine and milt were, however, much congested, and the muscle at the point of inoculation was soft and hæmorrhagic. In the bacteriological examination, *Micrococcus pyogenes* was obtained from the muscle at the site of inoculation, from the spleen and from the kidney. The muscle on the reverse side and the heart blood were sterile. Two fishes died on the ninth day, a Saturday, but owing to a misunderstanding regarding the necessity of a speedy despatch and the arrival at the laboratory on Sunday, the carcasses were not sent. A fourth trout, which had developed a white mark on the head, somehow eluded the protecting net and got out of the vessel. The caretaker noted that after death the mark had almost disappeared. It was unfortunate that this carcass was not despatched to me. A fifth fish died eight weeks after the inoculation with the external lesions of ophthalmia—viz., one of the eyes had decayed—only the capsule remained. The epidermis of the head and tail had whitened in places, but no ulcers had formed on the body. Upon dissection the organs were seen to be healthy, but there was a considerable congestion in the blood vessels of the intestine and body cavity. The organs, and especially the milt, were stained a deep orange-yellow by the bile. Like the fishes that died after natural infection, the body lesions of this experimental fish were not pronounced. The juices of the various organs, the blood and portions of the brain were examined bacteriologically, and while putrefactive bacteria were found, *Micrococcus pyogenes* could

neither be detected nor isolated. This is precisely what occurred with the fishes that succumbed naturally to the disease. It was only from those trout which had body sores that the micrococcus could be obtained. The sixth experimental trout was alive and apparently healthy at the time of writing.

In reviewing the infection experiment, it is seen that one was lost, thus reducing the number to five. Three of the five succumbed about the same time, and since the inoculated bacterium was recovered from one, it is probable that had the others been sent to the laboratory the organism would also have been recovered from them. The fourth developed ophthalmia and died, while the fifth was apparently unaffected by the inoculation.

Since the infected micrococcus could not be separated from the trout that succumbed with the lesion of ophthalmia, it is possible that the fish might have died quite independently of the inoculation. On enquiry, I was informed by Mr. Brodie that about three trout had died weekly from ophthalmia since the time of the inoculation, and that epidermal sores had never been observed after the height of the disease. As the tanks were carrying about 400 fishes, this means a death rate of 1 in 20 during the period that elapsed between the time of inoculation and the death of the fourth experimental fish.

So far as the infection experiments go, the micrococcus was pathogenic, although it did not produce lesions similar to those from which it was isolated. This can be explained by the experimental fishes being in more healthy surroundings. Had the water been as unhealthy as that in the tanks during the height of the disease, it is probable that the ulcers would have developed. Among mammals the same micrococcus produces sores when the vitality of the animal is lowered by some cause, and since it obtains among the higher animals, there is no reason why it should not also hold among fishes. Another point to be remembered is that by the time a constant flow of water could be depended upon at the hatchery about two months had passed, and during this time the bacterium may have lost in part its

ulcer-producing function while under artificial conditions of culture. There is, therefore, reason to believe that *Micrococcus pyogenes* is the cause of the disease in salmon trout, characterised by the production of ulcers, and that its specific pathogenic action is influenced by an unhealthy or low condition of the fish such as might be caused by an insufficient water supply and the overcrowding of the tanks.

There is the doubt that the production of sores and ophthalmia are induced by two different agents. The reasons for believing this are —(1) One carcase seldom shows both types of lesion; (2) fishes die of ophthalmia at times when no ulcers are found upon other trout; (3) *Micrococcus pyogenes* could not be isolated from those cases of ophthalmia which did not show the ulcers; and (4) the ulcer disease is known in other localities, and in describing the disease writers make no mention of ophthalmia.

In a report upon an epidemic among brook trout (*Salvelinus fontinalis*) on Long Island, Calkins\* describes a disease which appears to be identical with the ulcer disease of rainbow trout. His photograph of the diseased brook trout shows an appearance precisely similar to that which I saw on the rainbow trout. He did not examine the carcasses bacteriologically, but as the result of many observations upon sections of the organs of diseased fishes made after imbedding in paraffin, Calkins concluded that the disease was caused by a new sporozoon which he named *Lymphosporidium truttae*. The plates which are reproduced in his paper show spores  $2\ \mu$  in diameter, and he mentions sporozoites  $0.5\ \mu$  in diameter. The micrococci which I found might simulate these sporozoites. In the milt of diseased rainbow trout forms were met which at first sight appeared to be Calkins' spores, but these proved to be the bodies of the spermatozoa. The similarity between Calkins' spores and the bodies of the spermatozoa is remarkable. They both stain deeply with

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\* Fourth Annual Report of the Commissioners of Fisheries, Game and Forests of the State of New York, 1898.

nuclear stains; they are the same shape and the same size; and, lastly, they both have the cleft at the broad end, which Calkins says is the first stage in the formation of the sporozoites.

Marsh\* also writes about the same disease among brook trout, and mentions that Loch Leven trout are susceptible, but that rainbow trout, kept in ponds by the side of the Loch Leven trout, have not contracted the disease, and appear to be immune. The experience at Prospect shows that the immunity must have been accidental.

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\* The Fishing Gazette, xix 401, 417.